

**CONTROL OF BERMUDAGRASS (*Cynodon dactylon*) IN
ZOYSIAGRASS (*Zoysia japonica*) TURF BY USING POST-
EMERGENCE HERBICIDES**

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By
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CHAPTER I

Literature Review

Bermudagrass and Zoysiagrass General Information

Bermudagrass (*Cynodon dactylon* (L.) Pers.) and zoysiagrass (*Zoysia japonica* Steud.) are the two important warm-season turfgrasses in the southern region and transition zone of the United States (Shaver et al., 2006). Both species can be used on home lawns, recreational areas, roadsides, golf courses and other areas where a dense and low mowing height turf is desired. Recent improvements through breeding have dramatically promoted its usage in areas such as the Midwest, where winter-killing has been an issue. Some newly released cultivars, such as ‘Riviera’ and ‘Yukon’, for example, can even withstand snow and ice in the winter with minimum damage (Richardson et al., 2001).

In the 1950s, zoysiagrass quickly became one of the most widely spread grasses in golf courses in Midwest when the cultivar ‘Meyer’ was released (Grau and Radko., 1951). Due to its excellent heat, freezing, and drought tolerance in the transition zone, zoysiagrass has become one of the primary used species in the transition zone (Grau, 1952). Since then, many new cultivars of zoysiagrass are released later with improved texture, tolerance to biotic and abiotic stresses, and rapid growth rate compared to Meyer (Karcher et al., 2005; Patton et al., 2007; Reinert and Engelke, 2001; White and Engelke, 1990; White et al., 2001).

Although bermudagrass and zoysiagrass both provide ideal turf surfaces, a mixture of the two species results in unacceptable turf quality and reduced playability.

Compared to zoysiagrass, bermudagrass exhibits different texture and color, which disturbs the aesthetic value of zoysiagrass turf (Johnson, 1992). When growing out of place, bermudagrass is considered one of the toughest weeds to control, partially due to its extensive rhizomes and stolons, as well as aggressive growth habit (White and Busey, 1988). Additionally, zoysiagrass grows significantly slower compared to bermudagrass, which results in slow recovery from injuries such as disease or traffic (Karcher et al., 2005). All of these factors described above contribute to the persistence of bermudagrass when it encroaches in a zoysiagrass turf. Selective control of bermudagrass in zoysiagrass turf has been difficult, mainly because both of the species share similar physiological characteristics (Johnson, 1987). Both species develop stolons and rhizomes, and tolerate various mowing heights range from 10 cm down to several millimeters (Bunnell et al. 2005; Guertal and Evans, 2006; Salaiz et al. 1995). This explains why regular culture practices, such as mowing, are impossible to suppress bermudagrass on zoysiagrass turf. The two species also have a similar growth pattern in responses to seasonal changes. This leaves no other options for application on dormant zoysiagrass, which is otherwise an effective method to control weed populations on warm-season grasses including zoysiagrass. Additionally, zoysiagrass and bermudagrass share a similar sensitivity to most commonly used herbicides (Dernoeden, 1989; Johnson, 1992), and chemical applications typically result in unacceptable injuries to both species (White and Busey, 1988). The only remaining choice for turf managers is to renovate the entire area with non-selective herbicides such as glyphosate.

Control of Bermudagrass on Zoysiagrass Turf

Cultural Control

In the field, methods for control of bermudagrass include manual removal of rhizomes from the soil and deep plowing. Practices like plowing and raking help to move rhizomes to the surface where tissues dry out, thereby reducing viable rhizomes and stolons in the soil (Frans et al. 1982; Horowitz, 1996). However, when soil is wet, cultivation can propagate plants and spread them out, which leads to establishment of additional bermudagrass populations. Additionally, although cultivation can be effective in control of germinated plant tissues, this practice cannot limit the seeds that are left in the soil.

There are also other practices, which cannot control bermudagrass, but can help to maintain a healthy zoysiagrass turf and improve its ability to compete with most weeds including bermudagrass. Those practices include raise mowing height, avoid heavy fertilization in the summer, and mulch and edge to prevent bermudagrass from spreading out (Busey, 2003; Caamal-Maldonado et al., 2001; Crutchfield et al., 1986; Putnam et al., 1983; Skroch et al., 1992).

Chemical control

Half a century ago, chemicals like trichloroacetic acid and dalapon were used as the major products for common bermudagrass control in agriculture and horticulture areas (Dickens and Turner, 1984). Later in the 1970s, invention of glyphosate brought a huge impact to the agriculture world. Due to its excellent efficacy and safety to humans and the environment, glyphosate has quickly dominated the world herbicide market. Although effective on most weeds, control of bermudagrass is still a challenge; typically

three sequential applications of glyphosate are needed to obtain satisfactory (>95%) control of bermudagrass (Johnson, 1988). Additionally, due to its no-selective nature, injury to desirable turfgrass is inevitable (McCarty 1996). More recently, quizalofop, fluazifop and numerous post-emergence (POST) or pre-emergence (PRE) herbicides have been released to control common bermudagrass in agricultural and horticultural areas (Dickens and Turner, 1984).

Aryloxyphenoxypropionate (AOPP) herbicides have shown promise for bermudagrass control in zoysiagrass turf (McElroy, 2006). The target site of AOPP herbicides is the biotin-containing enzyme acetyl-coenzyme A carboxylase (ACCase), which is a crucial enzyme in the *de novo* fatty acid biosynthetic pathway (Inclendon et al., 1997). Fluazifop was first released in 1993. As a phenoxy chemical, fluazifop breaks down rapidly in moist soils and has less than one week's half-life (Hartley and Kidd, 1991), which indicates that application timing is critical for efficient control. Fluazifop is mainly used for selective POST control of annual and perennial grass weeds in soybeans and other broadleaf crops such as carrots (*Daucus carota* subsp. *Sativus*), spinach (*Spinacia oleracea*), potatoes (*Solanum tuberosum*), and ornamental plants. On zoysiagrass turf, application of fluazifop resulted in 69% bermudagrass control and only 5% zoysiagrass injury when tank mixed with triclopyr (McElroy and Breeden, 2006). Research conducted on 'Emerald' zoysiagrass (*Z. japonica* . X *Z. matrella* .. var. *tenuifolia*) showed up to 85% common bermudagrass suppression with 4 sequential fluazifop applications at 0.1 kg ha⁻¹, and control was maintained for two continuous years (Johnson, 1992). Meanwhile, fluazifop at higher rate than 0.1 kg ha⁻¹ did not result in an increased bermudagrass control, but caused up to 70% damage to the zoysiagrass

for 4 to 5 weeks. It was also found that additional applications of fluazifop more than two times per year did not improve bermudagrass control. Therefore, the author suggested that higher rate of fluazifop and multiple applications should be avoided as severe zoysiagrass damage is likely (Johnson, 1992).

Fenoxaprop-ethyl (fenoxaprop) is another primary AOPP herbicide evaluated for bermudagrass control in zoysiagrass (McElroy and Breeden, 2006). Multiple applications of fenoxaprop suppressed bermudagrass 80% or more (Bernoeden et al., 1989; Johnson et al., 1995). Other studies reported that fenoxaprop tank-mixed with triclopyr at 0.13 and 1.12 kg ha⁻¹, respectively, suppressed bermudagrass by 67 % with only 5% zoysiagrass injury (McElroy and Breeden, 2006). When applied at 0.63 kg ha⁻¹, fenoxaprop suppressed 80% bermudagrass with five sequential applications (Johnson and Carrow, 1995). When tank mixed with triclopyr at 1.4 kg ha⁻¹, sequential applications of fenoxaprop or fenoxaprop applied at 0.53 or 0.53 kg ha⁻¹, respectively also achieved a similarly bermudagrass control (Cudney et al., 1997). In a separated study, 63% and 51% control of ‘Tifway’ hybrid bermudagrass (*C. dactylon* x *C. transvaalensis*) was achieved by three sequential applications of fluazifop or fenoxaprop alone at 0.11 and 0.14 kg ha⁻¹, respectively, with ~20% injury on ‘Diamond’ (*Z. matrella*), ‘Palisades’ (*Z. japonica*), and ‘Zenith’ (*Z. japonica*) (Lewis et al. 2010). When tank mixed fluazifop or fenoxaprop with triclopyr, the control effects increased 78% and 76% with less injury on *Z. japonica* group at 10%.

Although AOPP herbicides like fluazifop have been widely used for POST grass weed control, one consequence of intensive use of AOPP herbicides is selection of herbicide-resistant weeds. Resistance in *Lolium rigidum* had become widespread in the

southern Australian cropping zone (Matthews, 1994). More recently, resistance has occurred in populations of oats (*Avena* spp.) in Australia and North America (Heap et al., 1993; Mansooji et al., 1992; Seefeldt et al., 1994). Therefore, it is likely that selection of herbicide-resistant weeds could occur on turf in the future.

Diclofop is a selective, systemic POST herbicide with contact action. It is mainly absorbed by the foliage, but also by the roots if soil is moist. Diclofop is used primarily for control of wild oats and annual grassy weeds found in numerous crops, like carrots, celery (*Apium graveolens* var. *dulce*), field beans (*Vicia faba*) and lettuce (*Lactuca sativa*) (Hartley and Kidd, 1991). Mechanisms of diclofop activity include depolarization of the membrane electronic potential, leading to an increase of plant cell proton permeability (Lucas et al., 1984; Wright, 1994). It is also an inhibitor of ACCase (Burton et al., 1989; Walker et al., 1988). Among MSMA (1.12 kg ha^{-1}), metsulfuron (0.021 kg ha^{-1}), diclofop (1.12 kg ha^{-1}), clopyralid (0.56 kg ha^{-1}), dicamba (0.56 kg ha^{-1}), 2, 4-D (0.56 kg ha^{-1}), and quinclorac (0.842 kg ha^{-1}), diclofop application caused the most severe injury to bermudagrass at injury ratings approaching 6 to 7 (0 to 9 scale where 9 means complete death) in a two years' field study and a greenhouse study. However, the injury was transient, and bermudagrass recovered within 30 days after treatment (McCalla, 2004).

Fluroxypyr and triclopyr (Tomlin, 2004) are two pyridine carboxylic acid chemicals registered as a POST herbicide for the control of broadleaf weeds and woody brush (Hartley and Kidd, 1991). The mode of action of pyridine carboxylic acid chemicals is similar to growth hormones which interfere with normal plant auxin growth processes (Retzinger and Smith, 1997). It can be absorbed by nearly any plant tissues like

green bark, leaves, roots, and cut stem surfaces and moves throughout the plant, before accumulates in the meristem tissues like stem and crown of the grass (United States Department of Agriculture, 1996). Triclopyr has been shown as a potential chemical to control bermudagrass when applied at twice the labeled rate (4.8 kg ha⁻¹) (Cudney et al., 1997). Multiple applications of triclopyr at 0.5 kg ha⁻¹ or triclopyr tank-mixing with clopyralid at 0.5 or 0.63 kg ha⁻¹, respectively, caused bermudagrass injury up to 30% (Johnson et al., 2001). A separate study found that when applied at 2 or 4 week intervals, fluazifop tank mixed with triclopyr caused lower zoysiagrass injury compared to fluazifop applied alone. Although the percent coverage was reduced to an unfavorable level (<78%), the data indicated that triclopyr had a protective effect when mixed with fluazifop (Lewis et al., 2008). Research conducted to control ‘Tifway’ bermudagrass on ‘Zorro’ zoysiagrass (*Z. matrella*) found that when tank mixed with clodinafop, fenoxaprop or metamifop, triclopyr significantly reduced the injury on ‘Zorro’ zoysiagrass from a maximum of 42% to nearly 0% (Dorah et al., 2011). On the other hand, ‘Tifway’ bermudagrass suppression was increased around 20% for all treatments. McElroy and Breeden (2006) found that when fluroxypyr or triclopyr was mixed with fenoxaprop or fluazifop, treatments contained triclopyr caused less injury to zoysiagrass (5% or less) than treatment contained fluroxypyr (up to 16%).

Mesotrione is mainly used as a broadleaf weed herbicide in turf, and can be applied both PRE and POST. The mode of action is inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) in target plants, leading to disruption of carotenoid biosynthesis in the chlorophyll pathway with bleaching symptoms (EPA, 2001). A study conducted to investigate the effect of mesotrione on ‘Tifway’

bermudagrass sod quality stated that mesotrione has very limited impact on 'Tifway' sod qualities like coverage and strength. Only the highest rate at 0.56 kg ha⁻¹ caused reduction of sod production, and the sod treated with label rates recovered in eight weeks and was ready for harvest with minimal injury (McCalla et al., 2010). Results indicated that the control effect on hybrid bermudagrass of mesotrione may be limited, but its effect on common bermudagrass is still unclear.

Metribuzin is a selective triazine herbicide which inhibits photosynthetic electron transport at the photosystem II receptor site in susceptible plant species (Extoxnet, 1996). It can be used for PRE and POST control of many grasses and numerous broadleaf weeds in crop, vegetable, and turfgrass areas. Two individual greenhouse and field experiments were conducted for weed control at bermudagrass establishment from seed. In both greenhouse and field studies, metribuzin was relatively safe to bermudagrass when applied as PRE (Fermanian et al., 1980). Richard (1993) stated that metribuzin application slowed bermudagrass spreading out into a sugarcane (*Saccharum* spp.) field. It was also reported that 8 weeks after metribuzin application as PRE applications, it provided better suppression (63% bermudagrass infestation) compared to atrazin (84% bermudagrass infestation) (Richard 1997).

Monosodium acid methanearsonate (MSMA) is used as a selective post-emergence herbicide to control crabgrass, yellow nutsedge and other annual grasses and broadleaf weeds. It is synthesized by complex reactions between methyl chloride and crude sodium arsenite. As a result, MSMA contains residuals of both sodium arsenite and sodium arsenate (Ford, 1999). Its production and sale have stopped since 2010 due to the potential toxicity of arsenic in inorganic form (Drexel Chemical Co., 2010). However,

users who have storages can still apply MSMA legally until they are exhausted. MSMA at 2.24 kg ha⁻¹ tank mixed with either methazole or metribuzin at 0.14 kg ha⁻¹ exhibited short term phytotoxicity on common and hybrid ‘Tifgreen’ bermudagrass (Murdoch and Ikeda, 1974). However, most discoloration recovered in 4 weeks after application. On zoysiagrass turf, MSMA applied at 14, 28 or 42 days after emergence provided excellent control of annual grassy weeds with acceptable zoysiagrass establishment. MSMA combined with dithiopyr applied 14 days after emergence caused 7% less zoysiagrass coverage compared to MSMA applied alone (Patton et al., 2004), which indicated a potential phytotoxicity on zoysiagrass. On seeded bermudagrass turfs, MSMA (2.24 kg ha⁻¹) tank mixture of metribuzin (0.64 kg ha⁻¹) caused unacceptable injury at 8 (0 to 9 scale where 9 means complete death) 2 WAT. However, the turf recovered in a month and no significantly coverage reduction was observed (Richardson et al., 2005).

Oxadiazon was registered in 1978 as a pre-emergence or early post-emergence herbicide for grassy and broadleaf weeds control. Its mechanism is interfering with the pathway of chlorophyll production which results in a breakdown of plant tissue when exposure to light (EPA, 2003). Oxadiazon also exhibited a growth regulation effects in bermudagrass and zoysiagrass. When applied as PRE, oxadiazon reduced the cold tolerance of ‘Tifway’ bermudagrass with approximately 14% less clipping and 47% less root growth compared to control (Breuninger et al., 1981). Another field test found that oxadiazon application enhanced zoysiagrass growth with more than 5 times coverage at 2 years after sprigging compared to control (Carroll et al., 1996).

Siduron was released in the United States in 1964 (EPA, 2008). It is a phenylurea herbicide registered for annual grassy weeds in newly seeded or established

cool season grasses. Its exact mode of action is still unknown but it is believed to inhibit some aspects of cell division, like mitosis disruption (EPA, 2008). Early research showed that when applied alone or following application of flurprimidol (0.4 kg ha^{-1}), mefluidide (0.1 kg ha^{-1}), or amidochlor (0.8 kg ha^{-1}), siduron (53.8 kg ha^{-1}) provided approximately 60% control on 'Tifway' bermudagrass with 10% injury on creeping bentgrass 6 WAT (Johnson et al., 1989). Furthermore, both foliar and stolon growth of three different bermudagrass cultivars, 'Tifway', 'Tifgreen', and 'Common', were significantly inhibited with acceptable bentgrass injury (~7%) from treatments contains siduron. De Mur (1973) conducted an experiment to test the environmental impact on the phytotoxicity of siduron. Results showed that bermudagrass was susceptible to siduron at only 1 particles per million (ppm), and shoot growth was more sensitive to siduron than root growth. This study also suggested that low light intensity decreased the toxicity of siduron to bermudagrass.

Other researchers found that the most effective way to remove bermudagrass was to apply non-selective herbicides. For instance, glyphosate tank mixed with fluazifop-p-butyl at 4.5 and 0.4 kg ha^{-1} , respectively, for at least three sequential applications at 4 weeks apart can completely remove bermudagrass (Teuton et al., 2005). Another highly recommended nonselective herbicide imazapyr was first released in the US in 1984, and had been proved its great effect on controlling weeds like white clover (*Trifolium repens* L.), mouseear duckweed (*Cerastium vulgatum* L.), and dandelion (*Taraxacum officinale* Wiggers) in warm season grass (Coats et al., 1986). It can be quickly absorbed by roots and leaves and distributed between these roots and foliage tissues. Imazapyr is an Acetolactate Synthase (ALS) inhibitor with the mechanism of

preventing production of amino acids valine, leucine, and isoleucine (DiTomaso 2002). Effects of this mechanism will slow down the synthesis of proteins and kill the growing points which further stop the growth of the whole plant. Imazapyr can be used to control common bermudagrass as well (Muzyk et al., 1986). In the sod field, researches conducted by Griffin (1994) and McCarty (1996) concluded that imazapyr obtained a great common bermudagrass control in centipedegrass, zoysiagrass, St. Augustinegrass and hybrid bermudagrass, although injury was observed in desired species. In the study conducted by Griffin (1994), imazapyr was applied at 0.8, 1.1, and 1.7 kg ha⁻¹ once, twice or three times at June 1, July 15, or September 1 with comparison to glyphosate applied at 2.2 kg ha⁻¹ at the same dates. Treatments were applied on pure stands of 'Tifway' bermudagrass and 'Emerald' zoysiagrass. All treatments repeated in two consecutive years and resulted in more than 90% control of the common bermudagrass. A single July application of imazapyr at 1.1 kg ha⁻¹ received 100% control on common bermudagrass. Results reported by Griffin (1994) indicated that single application of imazapyr resulted in less injury to 'Tifway' and 'Emerald' than that of multiple applications at the same total rate. However, the specific injury level on zoysiagrass is unclear. Ferrell et al. (2005) also reported a nearly 100% removal result from imazapyr applied alone at 0.56 or 1.12 kg ha⁻¹.

Plant growth regulators (PGRs) are man-made chemicals with similar compounds of plant hormones. As a result, PGRs can influence numerous plant physiological processes under very low concentrations (Helgi Öpik, 2005; Srivastava, 2002). A number of PGRs have been widely used for regulating the growth of cultivated plants or weeds. Trinexapac-ethyl is a gibberellin synthesis inhibitor which

suppresses plant growth by disrupting reactions from GA20 to GA1 (Rademacher, 2000). It inhibits the size of the plants without interfering with normal developmental processes. This results in continued limited development of new plant tissues with normal structure and function (Pannacci et al., 2004).

Trinexapac-ethyl is efficient in suppressing of various grass species. Field experiments conducted on evaluating the effect of trinexapac-ethyl on Kentucky bluegrass, perennial ryegrass, tall fescue, creeping red fescue (*Festuca rubra* ssp. *rubra* L.) and chewing fescue (*F. rubra* ssp. *commutata* Gaud.) growth found that at least two applications at the rate 0.375 kg ha⁻¹ were needed to affect vegetative growth in Kentucky bluegrass (40%), tall fescue (33%) and chewing fescue (21%) (Pannacci et al., 2004). In another study, compared with control plots, trinexapac-ethyl applied at 0.048 kg ha⁻¹ monthly or applied at 0.096 kg ha⁻¹ bimonthly on the ‘Diamond’ zoysiagrass turf resulted in 76 to 73% less shoot vertical growth and 77 to 75% less clippings, respectively (Qian et al., 1999). Treatments also resulted in 40 to 38% higher total nonstructural carbohydrates content, 60 to 50% higher roots mass, 51 to 46% higher root viability, and 48 to 42% more photosynthesis, respectively. A field study also resulted in a reduction of clippings by 40% and absolute sward height by up to 21% compared to untreated control (Daniels et al., 1996). Furthermore, trinexapac-ethyl has the capability to reduce grass vertical growth but strengthen horizontal growth of the shoots and vegetative tissues, leading to improved turf density and quality with reduced mowing requirement (McCullough et al., 2007). McCullough et al. (2006) found that in addition to an enhanced turf color, ‘TifEagle’ bermudagrass clippings were reduced 39% but root growth was promoted 23%. Research also suggested that trinexapac-ethyl application

enhanced the shade tolerance of zoysiagrass (Qian and Engelke, 1999) and creeping bentgrass (Goss et al., 2002), drought and heat tolerance of creeping bentgrass (McCann and Huang, 2007), and cold tolerance of bermudagrass (Richardson, 2002).

Ethephon, which promotes ethylene production, has a variety of effects on plants, including growth reduction and ripening acceleration (The Agrochemicals Handbook, 1983). Ethephon is mainly used in fruit production industry. On turf, ethephon stimulates stem elongation of common bermudagrass when applied at rates below 6.7 kg/ha in the greenhouse, while higher concentration reduced bermudagrass vegetative growth and seedhead development in the field (Wu et al., 1976). Other reports suggested that ethylene promotes the activity of indoleacetic acid in bermudagrass stolons (Balatti et al., 1989; Shatters et al., 1998), which enhances the plant growth and development by inducing cell elongation and cell division. McCullough et al. (2005) reported that when treated with ethephon at 3.8 and 7.6 kg ha⁻¹ every three weeks, turf quality of 'TifEagle' bermudagrass was reduced 33% at 9 weeks after initial application. Root mass of treated bermudagrass was also 20 and 30% lower than untreated turf after three applications. Meanwhile, root length was reduced 14 and 16%. Results indicate a negative impact of ethephon on hybrid bermudagrass growth.

Physiology Differences between Bermudagrass and Zoysiagrass

As C₄ plants, both bermudagrass and zoysiagrass have higher efficiency of CO₂ utilization than C₃ turf species, resulting in a higher growth rate and water usage efficiency (Brown, 1978). However, heat stress can be intensified by absence of surface air movement and excessive atmospheric humidity (Beard, 1995). Experiment conducted on evaluating bermudagrass anatomic changes under heat and drought stress (Utrillas and

Alegre, 1997) demonstrated that high temperature accompanied with water stress leads to both mesophyll and bundle sheath cells decline. Additional changes in bundle sheath cells include rearrangement of the thylakoids orientation and increase of starch in chloroplasts (Parker, 1963). Furthermore, peripheral reticulum and starch granules increased in chloroplasts, as well as the number of mitochondria in mesophyll cells. In contrast, the amount of grana stacks decreased, related to a decrease in leaf sodium concentration (Utrillas and Alegre, 1997). Consequently, prolonged stress period leads to significant reduction of chlorophyll content (Zhou and Abaraha, 2007).

In general, zoysiagrass has better cold tolerance than bermudagrass (Rogers et al., 1977). According to Rogers et al. (1977), zoysiagrass performed four to eight times more photosynthetic activities than that of bermudagrass under chilling condition. For example, cultivars like ‘Meyer’ and ‘Diamond’ zoysiagrass have the LD₅₀ ranged from -8.4 C to -11.5 C (Patten et al., 2007). Various research have conducted determine the influence of chilling stress on turfgrass carbohydrates, lipids, proteins, and proline concentrations (Cai et al., 2004; Cyril et al., 2002; Dionne et al., 2001; Fry et al., 1993; Patton et al., 2007; Thomas and James, 1993). These reports indicate that *Z. japonica* genotypes generally exhibit better cold tolerance and less freezing damage than *Z. matrella* genotypes. Additionally, seeded *Z. japonica* genotypes are more tolerant to freezing temperatures than other genotypes (Patton and Reicher, 2007). Research also found that *Z. matrella* cultivars, such as ‘Diamond’, ‘Royal’, and ‘Zorro’ are less tolerant to freezing temperature than the *Z. japonica* cultivars, such as ‘Victoria’ have poor tolerance to freezing temperature compared to *Z. japonica* genotypes ‘Meyer’, ‘Zenith’, ‘Palisades’, ‘El Toro’, ‘Companion’, and ‘J-36’ (Patton and Reicher, 2007). Zoysiagrass

'Meyer' is the best cold tolerance cultivar tested with LD₅₀ ranged from -11.1 to -12.8 C (Dunn et al., 1999; Rogers et al., 1975; Warmund et al., 1998; Zhang and Fry, 2006), whereas 'Emerald', 'El Toro', 'Cavalier', and 'Palisades' are tolerant to cold temperatures (Dunn et al., 1999). For this reason, 'Meyer' zoysiagrass has been extensively used in the transition zone (Dunn et al., 1999; Dunn and Diesburg, 2004).

Anderson et al. (1993) demonstrated that increased cold acclimation could improve freeze tolerance of many turfgrasses. The restore of adequate carbohydrates like starch and sucrose is one of the contributing factors for bermudagrass winter survival (Beard, 1973). Soluble sugars, such as sucrose also function as cryoprotectants which protect the cell integrity during the cold temperature. Additionally, the increase in nonstructural carbohydrates during cold acclimation has been reported in bermudagrass and other warm-season turfgrass species (Ball et al., 2002; Fry et al., 1993; Rogers et al., 1975; Shahba et al., 2003). Zhang et al. (2009) found that under freezing temperature, 90% of the polar lipids species in zoysiagrass were composed with digalactosyl diacylglycerol (DGDG), monogalactosyl diacylglycerol (MGDG), phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidic acids (PA). Additionally, the correlations between higher PC and abscisic acid (ABA) levels with lower LT₅₀ was observed in more cold tolerant cultivars. It is well known that the PC is the major component of cell membranes (Culli and DeKrukjff, 1979; Verleij et al. 1982; Welti et al., 2002).

Plant hormones like ABA are also known to play a significant role in stress resistance mechanism in many plant species (Chen and Gusta, 1983; Dörffling et al., 1990; Heino et al., 1990). Early reports indicated that ABA influences osmotic potential

and certain protein synthesis in freezing resistance of zoysiagrass (de los Reyes et al., 2001). Exogenous ABA application or environmental stresses like cold or drought also induced expression of a chitinase gene, CynCHT1 in bermudagrass (de los Reyes, 2001). Patton et al. (2007) found a dehydrin-like polypeptide was highly associated with freezing tolerance in zoysiagrass genotypes. However, the relationship between these genes and ABA is still unknown need to be further evaluated.

In comparison, zoysiagrass plants generally go dormant earlier than bermudagrass under water deficiency (Sifers et al., 1998). Bermudagrass also exhibits a better and quicker recovery rate than zoysiagrass after drought period (Sifers et al., 1998). Zoysiagrass also exhibits a higher evapotranspiration rate under both maximum evaporative demand (Beard et al., 1992) and normal field conditions (Green et al., 1991). Furthermore, compared to zoysiagrass, bermudagrass has a more rapid surface wax formation rate during increasing water stress (Kim et al., 1987), which helps the plant to hold more water under drought stress.

Summary

No selective herbicide can effectively control bermudagrass without injury zoysiagrass. Although lack of single selective herbicides, researches proved that treatments with low application rate and multiple applications in at least two consecutive years can obtain acceptable bermudagrass suppression with less zoysiagrass injury. It is important to point out that application timing significantly influences the control effect in previous studies. As zoysiagrass usually needs more irrigation to maintain activity in heat and drought stress but has a better cold tolerance than bermudagrass, environmental conditions need to be considered when determines the application timing.

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CHAPTER II

Differential Responses of Bermudagrass (*Cynodon dactylon*) and Zoysiagrass (*Zoysia japonica*) Cultivars to Various Herbicides and PGRs

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Abstract: Bermudagrass (*Cynodon dactylon* (L.) Pers.) and zoysiagrass (*Zoysia japonica* Steud.) are the two most important warm-season turfgrasses. With the development of cold-tolerant bermudagrasses, there is a growing problem with bermudagrass encroaching into zoysiagrass in the northern transition zone. This study was conducted to determine the species and variety response variations of bermudagrass and zoysiagrass to various herbicides and plant growth regulators (PGRs). ‘Quickstand’ and ‘Riviera’ bermudagrass, and ‘El Toro’ and ‘Meyer’ zoysiagrass were planted in the greenhouse and fifteen different herbicides and PGR treatments were applied to the plants in three different studies. Factorial treatment combinations of species and variety and chemical treatments in addition to untreated controls were arranged in a randomized complete block design with 4 replications in each study. Chemical treatments included fenoxaprop, fluazifop, fluroxypyr, mesotrione, triclopyr, metribuzin, MSMA, oxadiazon,

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trinexapac-ethyl, ethephon and various combinations. Treatment effects were demonstrated as discoloration and stunted growth, and were measured weekly as percent discolorations and clipping biomass productions for up to 8 weeks after treatment (WAT). Root biomass was also collected at 8 WAT. It was found that except for root biomass, most chemicals evaluated resulted in significant variations between and/or within the two species. Aryloxyphenoxypropionate (AOPP) herbicides fenoxaprop and fluazifop caused greater discoloration and growth reduction to bermudagrass than zoysiagrass. Among the two bermudagrasses, 'Quickstand' was more sensitive to the AOPP herbicides than 'Riviera', with treatments causing 2~7 times more discoloration. A similar trend was also found for the treatment containing metribuzin at 0.2 kg ai ha⁻¹, where discoloration of 'Quickstand' and 'Riviera' bermudagrasses were ~15 or 8 times greater than 'Meyer' zoysiagrass, respectively. Metribuzin also reduced the growth rate of the two bermudagrass cultivars by ~60%, but had no effect on the zoysiagrass cultivars. This study suggested that inter- and intra-species variations need to be considered before developing a turfgrass herbicide program to control bermudagrass encroachment into zoysiagrass.

Nomenclature: Bermudagrass, *Cynodon dactylon*; zoysiagrass, *Zoysia japonica*.

Keywords: Fenoxaprop; fluazifop; plant growth regulator; discoloration.

Introduction

Bermudagrass (*Cynodon* spp.) and zoysiagrass (*Zoysia* spp.) are the two most commonly used warm-season grasses in the southern area and transition zone of the United State (Shaver et al., 2006). Although the two species have different colors and textures, bermudagrass and zoysiagrass are both popular in various landscapes, including home lawns, parks, golf courses and other turf areas (Johnson, 1992). In the past, zoysiagrass, especially Japanese lawn grass (*Z. japonica*), was more adapted to northern transition zone since it was generally more cold tolerant than bermudagrass (Patton and Reicher, 2007; Patton et al., 2007; Rogers et al., 1977). Recent developments of cold tolerant common bermudagrass (*C. dactylon*) (Richardson et al., 2001) have extended its adaptation further north. When the two species mix, however, it reduces turf quality and playability (Johnson, 1992). With the increasing reports of one species encroaching into another species (McElroy and Breeden, 2006; Johnson, 1992), it is important to investigate how the two species respond to commonly used herbicides.

Bermudagrass and zoysiagrass respond similarly to most herbicides (Johnson, 1987). However, research has found that the herbicide family aryloxyphenoxypropionate (AOPP), such as fenoxaprop and fluazifop, causes more phytotoxicity to bermudagrass than zoysiagrass and hence can be used to selectively suppress bermudagrass on zoysiagrass turf (McElroy and Breeden, 2006; Lewis et al., 2010; Doroh et al., 2011). In addition to AOPP herbicide, little research has investigated the potential variations of the two species in responding to other commonly used herbicides and plant growth regulators (PGRs).

Genetic variations exist among different cultivars of the same species. Previous research found that common bermudagrass cultivars ‘Princess 77’, ‘Riviera’, ‘Savannah’, and ‘Yukon’ showed significantly different injuries and growth responses to various herbicides such as atrazine, 2,4-D, clopyralid, dicamba, MCPP, foramsulfuron, MSMA, quinclorac, triclopyr, and trifloxysulfuron (McElroy et al., 2005). Similarly, variations were also found in zoysiagrass varieties in response to similar chemicals (Johnson 1978; Johnson and Carrow 1999). Flessner et al. (2011) reported significant differences among ‘Empire’ and ‘Meyer’ (*Z. japonica*), ‘Cavalier’ and ‘Zorro’ (*Z. matrella*), and ‘Emerald’ and ‘BK-7’ (*Z. japonica* X *Z. pacifica*) in response to aminocyclopyrachlor (AMCP) herbicide. Therefore, the objective of this research was to investigate differential responses of inter- and intra-species variations of *Z. japonica* and *C. dactylon* in responding to commonly used turfgrass herbicides and PGRs.

Materials and Methods

Three greenhouse studies were conducted at the University of Missouri with five chemical treatments in addition to the untreated control (Table 2.1.). Two bermudagrass cultivars, ‘Riviera’ and ‘Quickstand’, and two zoysiagrass cultivars, ‘Meyer’ and ‘El Toro’ were included in each study. All plants were growing in pots with 13 cm diameter and depth with Pro-mix (Premier Tech Horticulture, Quakertown, PA) potting soil. Plants were maintained with adequate irrigation and weekly fertilizations at 12.25 kg N ha⁻¹, and maintained at 3.2 cm cutting height. Greenhouse temperature was set at 25 °C, with variations ranged from 13 to 40 °C in different seasons. Overhead lights were provided with 14 hours photoperiod with light intensity ~ 800 μmol s⁻¹ m⁻². The three studies were conducted sequentially with each initiated in January, April, and

September 2010 for study 1, 2, and 3, respectively, and terminated at 8 weeks after the treatment (WAT). All treatments were tank-mixed with a non-ironic surfactant at 0.25% (v/v). Treatments were applied by an air-driven hydraulic sprayer calibrated to deliver 140 L ha⁻¹ at a spray pressure of 234 kPa using TeeJet XR8001 even flat fan nozzles (TeeJet Technologies, Springfield, Illinois).

Treatment effects were evaluated weekly as percent discoloration (%) and clipping biomass (g). Discoloration mainly appeared to be yellowing, and was visually assessed and recorded in a 0 to 100% scale with 0% indicates no discoloration and 100% indicates complete discoloration. To better present the treatment effect during entire period of each experiment, accumulated injury was calculated based on the following equation:

$$AUPIC = \sum_{i=1}^{n-1} ((X_{i+1} + X_i) / 2) \times (t_{i+1} - t_i) \quad (1)$$

where AUPIC represents the area under percentage injury curves, X_i = percent discoloration at i th observation, t_i = days after treatments at the i th observation, and n = number of total observations (Campbell and Madden, 1990). A lower AUPIC value indicates less discoloration for certain chemical treatment.

Treatment effects on turfgrass growth were assessed by clipping biomass productions. Plants were cut weekly and clippings were collected and oven dried at 70 °C for 72 hours and dry biomass was recorded. Total clipping biomass during the entire 8 weeks period were added together and compared to the untreated control for each species/cultivar, and presented as percent clipping biomass (PCB, %). At 8 WAT, root

samples were collected and removed from soil under tap water. The root biomass was recorded after oven drying at 70 °C for 72 hours.

The experimental design of the three greenhouse studies was a randomized complete block design with 4 replications. Two cultivars in each species and five chemical treatments in addition to an untreated control were arranged in a factorial treatment combination with total 24 treatments for each study. Analysis of variance was conducted by PROC MIX program of SAS 9.2 (SAS Institute, Cary, NC). Mean separation was conducted based on Fisher's Protected LSD at $P = 0.05$.

Result and Discussion

Treatment Effects on Color Maintenance

Symptoms of treatment injury included different levels of discoloration to the above ground tissues. A representative image of discoloration after tank-mixing of fenoxaprop and triclopyr at 2 WAT is presented in Figure 2.1 The accumulated effects of discoloration are presented as AUPIC in Figure 2.2A, 2.2B, and 2.2C for study 1, 2, and 3, respectively. Higher AUPIC values indicated higher levels of discoloration over the 8 week period of each study. Interactions of treatment \times cultivar were found in all studies, and hence two LSD values were presented in each study for the comparison among cultivars within each treatments (LSD bar a) and among treatments within each cultivars (LSD bar b) (Figure 2.2). Treated plants exhibited significant inter- and intra-species variations in discoloration caused by various chemicals applied.

All treatments including AOPP herbicides in study 1 caused significant discoloration to the two bermudagrass varieties but minimal impact on zoysiagrass (Figure 2.2A). AOPP herbicides, including fenoxaprop or fluazifop, alone or in tank-

mixture with triclopyr have been used effectively for selective control of bermudagrass on zoysiagrass turf (McElroy and Breeden, 2006). Compared to AOPP herbicides alone, tank-mixing with triclopyr reduced discoloration to zoysiagrass, especially ‘Meyer’ zoysiagrass. After treatment of tank-mixture of fluazifop and triclopyr, discolorations of ‘Meyer’ declined from 10% or higher by fluazifop alone to ~0%, which was ~400% changes in total AUPIC during the 8 week period. This result supported the previous report that tank-mixing with triclopyr reduced the injury of fenoxaprop or fluazifop to ‘Palisades’ and ‘Zenith’ (*Z. japonica*) zoysiagrass by ~60% (Lewis et al., 2010). Significant differences existed between the two bermudagrass cultivars treated by AOPP containing treatments, with ‘Quickstand’ resulting in 2 to 7 times greater discoloration than ‘Riviera’ during the 8 week period. Differential responses due to genetic variations among cultivars in the same species have been reported. McElroy et al. (2005) found a significant variation in injury symptoms among ‘Princess 77’, ‘Riviera’, ‘Savannah’, and ‘Yukon’ bermudagrass when treated with atrazine, 2,4-D, clopyralid, dicamba, MCPP, foramsulfuron, MSMA, quinclorac, triclopyr, and trifloxysulfuron at different rates and combinations. These results may provide an explanation to inconsistent bermudagrass control among various studies when different bermudagrass species/cultivars were involved (Doroh et al., 2011; Lewis et al., 2010; McElroy and Breeden, 2006). Additionally, ‘Riviera’ bermudagrass appeared to be equally sensitive to the either fenoxaprop or fluazifop containing treatments. On the other hand, ‘Quickstand’ bermudagrass was more sensitive to fenoxaprop with nearly 2 times more discoloration caused by fenoxaprop containing treatments than treatments containing fluazifop. The mechanism of why certain bermudagrass variety was more sensitive to specific AOPP

herbicide is unknown and worthy for future study. Treatment with fluroxypyr, however, did not cause significant discolorations to all tested species/varieties (Figure 2.2A.). This result was similar to the early report by McElroy and Breeden (2006), who found that single fluroxypyr applications caused no injury to ‘Meyer’ and ‘Cavalier’ (*Z. matrella*) zoysiagrass, and only 9% injury on a common bermudagrass.

Similarly, treatments in study 2 caused significant discoloration to ‘Quickstand’ and ‘Riviera’ bermudagrass (Figure 2.2B). In comparison, zoysiagrass, especially ‘El Toro’, was not affected by treatments containing mesotrione or metribuzin. This result did not agree with a field-based study where mesotrione treatments caused unacceptable discoloration to zoysiagrass. The mode of action of mesotrione is the inhibiting of the plastid enzyme *p*-Hydroxyphenylpyruvate dioxygenase (HPPD) in target plants, leading to disruption of carotenoid and chlorophyll biosynthesis (EPA, 2001). Symptoms of sensitive plants include foliage devoid of all pigments (white), with plants not capable of photosynthesis (Hess 2000). HPPD herbicide efficacy is enhanced by high light intensity with excess energy, with oxygen radicals accumulating and destroying chlorophyll molecules (Meazza et al., 2002; Hess, 2000). Compared to the field conditions, lower light intensity inside the greenhouse may explain the low levels of discoloration of zoysiagrass observed in this study. Additionally, bermudagrass and zoysiagrass showed differential responses to metribuzin. Compared to ‘Meyer’ zoysiagrass, discolorations of ‘Quickstand’ and ‘Riviera’ bermudagrass were ~15 or 8 times greater, respectively (Figure 2.2B). Metribuzin is a triazine herbicide which inhibits photosynthetic electron transport at the QB polypeptide in the photosystem II receptor site of susceptible plant species (Huppat, 1996). This herbicide was found to be relatively non-toxic to

bermudagrass when applied as pre-emergence (Fermanian et al., 1980). However, when applied post-emergence, metribuzin was found to reduce bermudagrass infestations up to 63% at 8 WAT (Richard, 1997). Our results confirmed the variation between the two species, where bermudagrass is more sensitive to metribuzin than zoysiagrass.

Tank-mixtures of MSMA and metribuzin caused the most severe injury to both species among all other treatments in study 3. A higher rate of MSMA at 6.6 kg ai ha⁻¹ resulted in significantly greater discolorations to all tested plants than the lower rate at 2.24 kg ai ha⁻¹ (Figure 2.2C). Similar results were observed by Richardson et al. (2005), who found that at 2 WAT tank-mixing of MSMA (2.24 kg ai ha⁻¹) and metribuzin (0.64 kg ai ha⁻¹) caused severe injury to bermudagrass at a level of 8 using a 1 to 9 scale, where 9 indicates total death. However, unlike metribuzin alone in study 2, both of the species were injured by treatment combinations of metribuzin and MSMA. Treatment with a commonly used pre-emergence herbicide, oxadiazon, resulted in >500 AUPIC to all tested species/cultivars with significantly greater impact on bermudagrass (Figure 2.2C). Previous research has found that single applications of oxadiazon (4.5 kg ai ha⁻¹) at different months from April to September significantly reduced the spring green-up and vegetative growth of ‘Tifway’, ‘Tifgreen’, ‘Tifdwarf’, and ‘Ormond’ (*Cynodon* spp.) bermudagrass in the following season (Johnson, 1976; Johnson, 1985). Our results agreed with the earlier findings that oxadiazon causes certain phytotoxicity to mature turfgrass species including bermudagrass.

Two commonly used PGRs were also evaluated in study 3 and our results found that trinexapac-ethyl had minimal impact on zoysiagrass colors (Figure 2.2C). However, the two bermudagrass cultivars were more sensitive to trinexapac-ethyl, with ~7 or 11

times greater discoloration found for ‘Quickstand’ and ‘Riviera’, respectively. In addition to inhibiting shoot growth of turfgrass species (Fagerness et al., 2002; McCullough et al., 2006; Qian and Engelke, 1999), trinexapac-ethyl has been reported to cause limited and transient discoloration to sensitive species, usually within 4 WAT (Fagerness et al., 2002; McCullough et al., 2006). On the other hand, ethephon, another commonly used PGR, had nearly no impact on the color maintenance of the two species (Figure 2.2C).

Treatment Effects on Shoot Growth Maintenance

In addition to tissue discoloration, treatment effects were also observed as stunted growth. Weekly clipping biomass was recorded and added together during the 8 week period, and presented as PCB (%) productions for each study (Figure 2.3). There were significant interactions of treatment × cultivar, and hence two LSD bars were presented for comparisons among cultivars within each treatment (LSD bar a) and among treatments within each cultivar (LSD bar b). As expected, significant variations existed between/within species, and among different chemical treatments (Figure 2.3).

Treatments containing AOPP herbicides reduced bermudagrass growth by ~50% or more, regardless of tank-mixing with triclopyr (Figure 2.3A). In comparison, the impact of AOPP herbicides on zoysiagrass growth is relatively less than bermudagrass. After tank-mixing with triclopyr and AOPP herbicides, both of the two zoysiagrass cultivars maintained the same growth rate compared to the control plants, with a PCB value $\geq 100\%$. Collectively, these results along with the discoloration presented in Figure 2.2A indicated that tank-mixing of triclopyr with AOPP herbicides improved AOPP herbicide safety on zoysiagrass. This conclusion is supported by reports where triclopyr significantly improves safety of AOPP herbicides on zoysiagrass (Lewis et al., 2010;

McElroy and Breeden, 2006). However, our results showed no evidence that tank-mixing triclopyr with AOPP herbicides increased the efficacy on bermudagrass control. This result was different from previous reports, where >10% increases in bermudagrass control were achieved after triclopyr tank-mixed with fenoxaprop or fluazifop (Doroh et al., 2011; Lewis et al., 2010). Treatment with fluroxypyr did not impact growth of either bermudagrass or zoysiagrass compared to untreated controls. Collectively, our results indicated that at the 0.23 kg ai ha⁻¹, fluroxypyr can be used safely on both of the two warm-season species. At higher rates, however, single application of fluroxypyr at 0.42 kg ai ha⁻¹ reduced 'Coastal' bermudagrass (*C. dactylon*) biomass to 75% at 4 months after treatment (Butler and Muir, 2006). Therefore, there might be a dosage effect for bermudagrass in responding to fluroxypyr.

The growth rate of both bermudagrass and zoysiagrass was reduced to ~60% or less after treatment with imazapyr in study 2 (Figure 2.3B.). As a non-selective herbicide, early research has found that bermudagrass can be effectively removed (up to 100%) by imazapyr treatment at 1.1 kg ai ha⁻¹ (Ferrell et al., 2005; Griffin et al., 1994). However, our study found that zoysiagrass was sensitive to imazapyr at 0.2 kg ai ha⁻¹ as well. Other treatments with mesotrione resulted in <25% growth reduction compared to untreated plants, regardless of the species or cultivars. Our results indicated that although mesotrione treatments caused greater discolorations to bermudagrass (Figure 2.2B), the treatment effect on growth reduction between bermudagrass and zoysiagrass were comparable. This result may explain the observations from a field study where mesotrione containing treatments failed to selectively suppress bermudagrass on a zoysiagrass turf, despite the variation in discoloration. Similarly, an early study also

found that mesotrione at 0.28 or 0.56 kg ai ha⁻¹ showed limited injury on ‘Tifway’ hybrid bermudagrass (*C. dactylon* x *C. transvaalensis*) where an injury level of 4 on a 1 to 9 scale was observed (McCalla et al., 2010). On the other hand, treatment with metribuzin caused no growth reduction to the two zoysiagrass cultivars compared to the untreated plants, but resulted in ~40% reduction to the two bermudagrass cultivars (Figure 2.3B). Collectively, the stunting and discoloration results indicated that bermudagrass was sensitive to metribuzin, but zoysiagrass was not affected by this herbicide. Previous research has found similar results on bermudagrass suppression (Richard, 1997), and hence metribuzin could be an alternative herbicide for selectively suppressing bermudagrass on zoysiagrass turf.

When tank-mixing metribuzin with MSMA, however, both bermudagrass and zoysiagrass growth were significantly reduced, with higher MSMA rates further reducing clipping production (Figure 2.3C). Compared to the two zoysiagrass cultivars, treatment with oxadiazon significantly reduced the growth rates of the two bermudagrasses, especially ‘Riviera’ bermudagrass where ~55% growth reduction was found. This result corresponded to the discoloration results presented in Figure 2.2B, where greater discolorations were found in ‘Riviera’ than ‘Quickstand’ bermudagrass. When PGRs were considered, trinexapac-ethyl significantly reduced the growth of all tested turfgrass species/cultivars as expected, while the influence of ethephon was minimal. The only exception was ‘Riviera’ bermudagrass, which showed an 83% growth reduction by ethephon application.

Treatment Effects on Root Biomass

Despite the treatment effects on the above ground tissues of bermudagrass and zoysiagrass, treatments applied in all three studies did not significantly affect root biomass collected at 8 WAT. Therefore, root biomass of individual cultivars was pooled across all treatments and mean separations were conducted when cultivar effects were significant (Table 2.2.). ‘El Toro’ zoysiagrass produced significantly more root biomass than ‘Meyer’ in study 1 and 3, and showed a similar trend in study 2. Compared to the two bermudagrass cultivars, no differences in root biomass were found between ‘Quickstand’ and ‘Riviera’. However, when compared to zoysiagrass, the two bermudagrass cultivars produced ~27% or less root biomass in study 3. It is likely that study 3 was initiated towards the end of the growing season in middle September when bermudagrass is more sensitive to environmental changes such as reduced temperature, photoperiod and light intensity. Burns (1972) observed 67 and 42% less root branching of bermudagrass (*Cynodon* sp. var. FB-137) at low temperature or under a low light intensity. Similarly, Schmidt and Blaser (1969) reported a significantly reduced top-root development and total root production at lower temperature (12°C) compared to higher temperatures (16 to 36 °C). Our results suggested that at 8 WAT, root growth was not affected by all treatments applied in the three studies, but was more a function of genetic and environmental factors.

In summary, there were significant inter- and intra-species variations of bermudagrass and zoysiagrass in responses to various herbicides and PGRs. Effects of chemical treatments appeared to be discoloration and stunting to sensitive species/cultivars, but not root growth at 8 WAT. Significant intra-species variations may

contribute to the inconsistent results in responses to various chemicals, and therefore the end users are recommended to consider the genetic variations within the same species when developing a chemical program.

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Table 2.1. Herbicides and plant growth regulators (PGRs) applied in three greenhouse studies to evaluate the differential responses of ‘Meyer’ or ‘El Toro’ zoysiagrass and ‘Quickstand’ or ‘Riviera’ bermudagrass.

Treatments	Chemicals	Rate (kg ai ha⁻¹)
----- Study 1 -----		
1	Fenoxaprop	0.2
2	Fenoxaprop + triclopyr	0.14 + 0.23
3	Fluazifop	0.2
4	Fluazifop + triclopyr	0.09 + 1.00
5	Fluroxypyr	0.23
6	Control	--
----- Study 2 -----		
7	Imazapyr	0.2
8	Mesotrione	0.175
9	Mesotrione + fenoxaprop	0.175 + 0.14
10	Mesotrione + fluazifop	0.175 + 0.09
11	Metribuzin	0.2
12	Control	--
----- Study 3 -----		
13	Metribuzin + MSMA	0.42 + 2.24
14	Metribuzin + MSMA	0.42 + 6.6
15	Oxadiazon	3.4
16	Trinexapac-ethyl	0.04
17	Ethephon	3.8
18	Control	--

Table 2.2. Root biomass (g) of two zoysiagrass cultivars ‘El Toro’ and ‘Meyer’ and two bermudagrass cultivars ‘Quickstand’ and ‘Riviera’ influenced by various chemical applications at 8 weeks after treatment (WAT) in three individual greenhouse studies^a.

Cultivars	Root biomass (g)		
	Study 1	Study 2	Study 3
El Toro	5.19 a ^b	4.55 ^c	4.70 a
Meyer	4.23 b	3.94	3.69 b
Quickstand	4.06 b	4.29	2.56 c
Riviera	4.39 ab	4.45	2.89 c

^a No significant chemical treatment effects were found and hence results were pooled across all treatments for each cultivar in each study.

^b Means within each study labeled by the same letters are not significantly different according to Fisher’s Protected LSD ($P = 0.05$).

^c No significant differences of root biomass were found in study 2 and hence mean separation were not conducted.

Figure 2.1. Discoloration of two zoysiagrass cultivars ‘El Toro’ and ‘Meyer’ and two bermudagrass cultivars ‘Riviera’ and ‘Quickstand’ (rows 1, 2, 3 and 4 from top to bottom, respectively) in responses to tank-mixing of fenoxaprop with triclopyr at 2 WAT. The first column on the left was the untreated controls for each cultivar, and the rest of the four columns were treated plants.



Figure 2.2. Area under percentage injury curves (AUPIC) of ‘El Toro’ (ET) and ‘Meyer’ (MY) zoysiagrass and ‘Quickstand’ (QS) and ‘Riviera’ (RV) bermudagrass in responses to various chemical treatments. Results of the three studies were presented in separated graphs with A: study 1; B: study 2; and C: study 3. For comparisons between cultivars or across treatments within each cultivar, means within the LSD bar (a) or LSD bar (b) are not significantly different based on Fisher’s Protected LSD at $P = 0.05$.

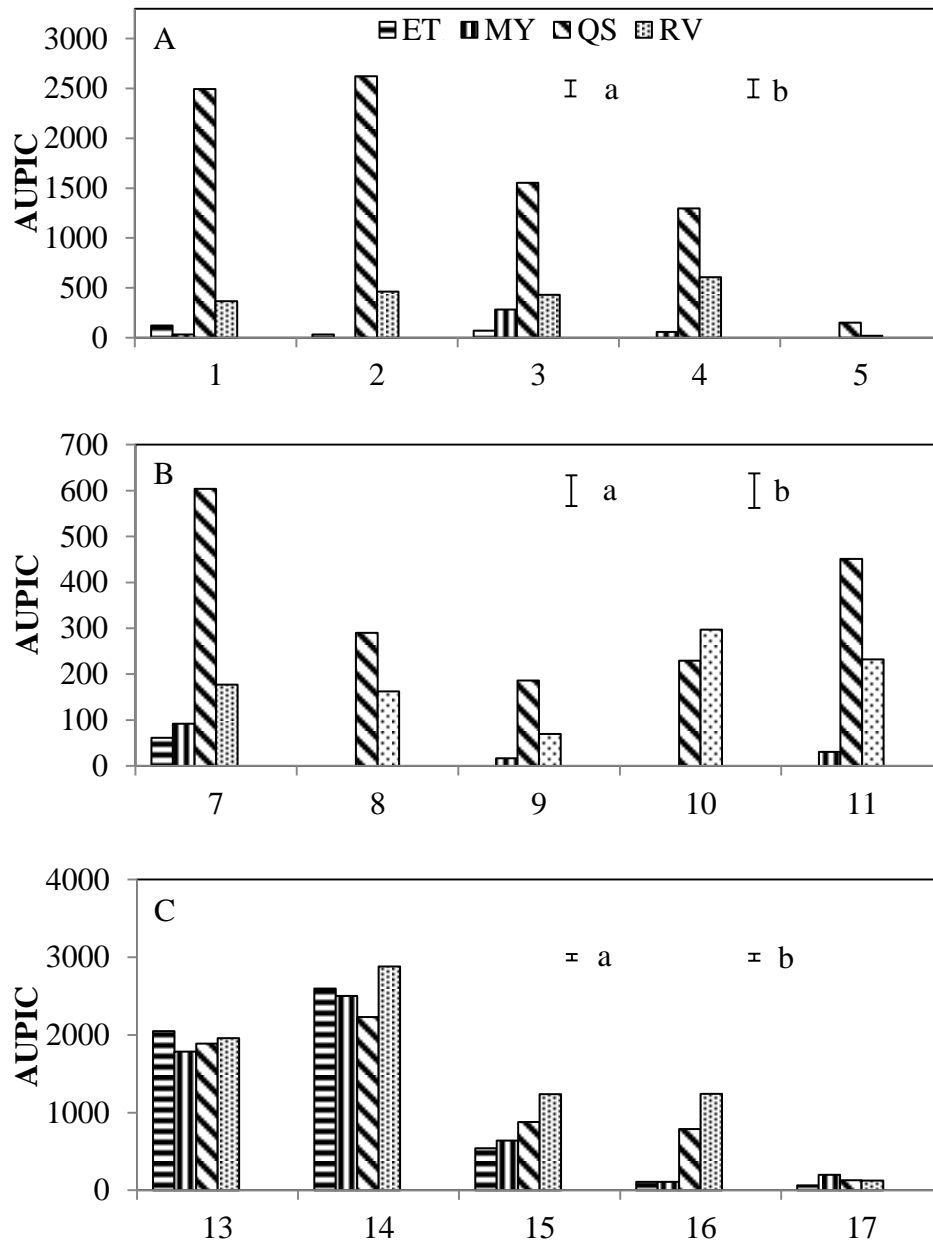
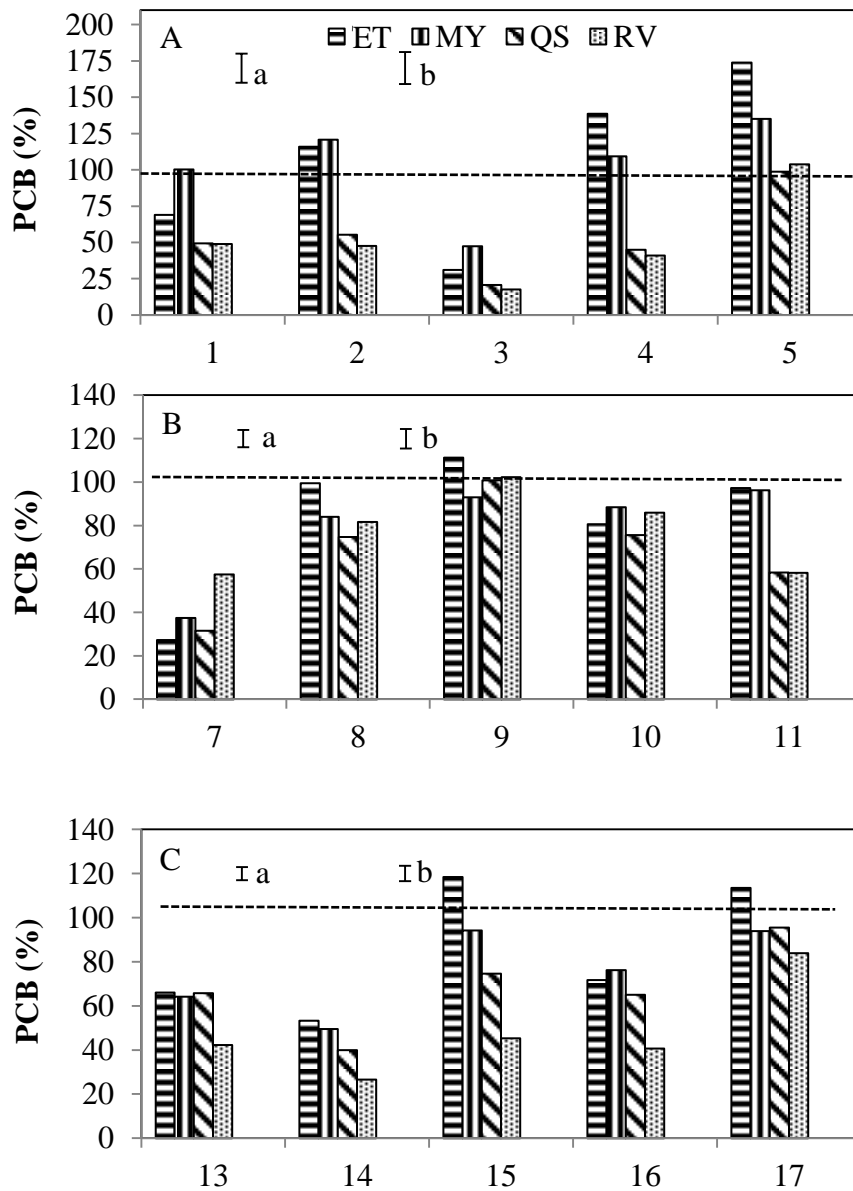


Figure 2.3. Percent accumulated clipping biomass (PAB) (%) of ‘El Toro’ (ET) and ‘Meyer’ (MY) zoysiagrass and ‘Quickstand’ (QS) and ‘Riviera’ (RV) bermudagrass.

Results of the three studies were presented in separated graphs with A: study 1; B: study 2; and C: study 3. For comparisons between cultivars or across treatments within each cultivar, means within the LSD bar (a) or LSD bar (b) are not significantly different based on Fisher’s Protected LSD at $P = 0.05$. 100% PCB lines were given in each graph.



CHAPTER III

Effect of Traffic Stress and Aryloxyphenoxypropionate Herbicides on Control of Common Bermudagrass (*Cynodon dactylon*) in a Zoysiagrass (*Zoysia japonica*) Fairway

Enzhan Song and Xi Xiong*

Abstract: Golf course superintendents in Missouri and surrounding states struggle to limit common bermudagrass (*Cynodon dactylon* (L.) Pers.) infestations in zoysiagrass (*Zoysia japonica* Steud.) fairways. Aryloxyphenoxypropionate (AOPP) herbicides tank-mixed with triclopyr effectively control hybrid bermudagrass (*C. dactylon* × *C. transvaalensis*) with minimal injury to zoysiagrass. However, limited research has focused on control of common bermudagrass in a zoysiagrass fairway where both species exist. A field study was conducted on a fairway where previous renovation of bermudagrass to zoysiagrass resulted in bermudagrass encroachment. Plots were established on two locations with traffic stress characterized as high traffic (HTA) or low traffic (LTA) areas. Treatments including AOPP herbicides tank-mixed with triclopyr or mesotrione were applied repeatedly over two seasons to the same plots. Applications of

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AOPP herbicides tank-mixed with mesotrione caused severe injury to zoysiagrass. Traffic stress provided a competitive advantage to bermudagrass that its coverage in untreated plots expanded more than 2-fold in HTA compared to LTA during the two growing seasons. The most effective treatment was fluazifop tank-mixed with triclopyr at 0.09 kg ai ha⁻¹ and 1.0 kg ae ha⁻¹, respectively, which resulted in 100% bermudagrass control and a smooth transition without compromising turf quality.

Nomenclature: Bermudagrass, *Cynodon dactylon*; hybrid bermudagrass, *C. dactylon* × *C. transvaalensis*); zoysiagrass, *Zoysia japonica*.

Keywords: Fenoxaprop; fluazifop; area under percent bermudagrass curve; normalized difference vegetation index.

Introduction

Bermudagrass (*Cynodon* spp.) and zoysiagrass (*Zoysia* spp.) are commonly used in golf course fairways in the southern and transition zones (Lyman et al., 2007). Although both species are warm-season grasses, they exhibit different colors and textures, and a mixture of the two species often results in reduced turf quality and playability (Dernoeden 1989; Johnson 1992). In states located at the northern transition zone, common bermudagrass (*C. dactylon*), such as ‘Westwood’, ‘Quickstand’, and ‘U3’, were once popular grasses for golf course fairways (Elmore et al., 2011). However, after repeated winter-kill events in the 1990s, a majority of the golf courses in Missouri renovated fairways to ‘Meyer’ zoysiagrass (*Z. japonica*) (Foy 2001). Despite the renovation efforts, many superintendents in this region reported the encroachment of common bermudagrass with coarse leaves, thick and long stolons, and an aggressive growth habit.

Bermudagrass is a difficult weed to control. Typically three sequential applications of glyphosate are required to obtain satisfactory (>95%) control (Teuton et al., 2005). Consequently, a costly renovation is necessary to transition bermudagrass to zoysiagrass on a golf course fairway (Foy 2001). Previous research shows that aryloxyphenoxypropionate (AOPP) herbicides, such as fenoxaprop and fluazifop, are promising for selective control of bermudagrass in various warm- and cool-season grasses (Cudney et al., 1997; Dernoeden 1989; Johnson 1992; Johnson and Carrow, 1995). Research further indicates that triclopyr alone (Bell et al., 2004) or tank-mixed with AOPP herbicides (Brosnan et al., 2011b; Doroh et al., 2011; Lewis et al., 2010; McElroy and Breeden, 2006) are effective for bermudagrass control with minimal injury

on zoysiagrass. However, earlier research was conducted either on monoculture stands of zoysiagrass or bermudagrass (Lewis et al., 2010; McElroy and Breedon, 2006), or on a zoysiagrass turf with pieces of sod replaced by bermudagrass (Brosnan et al., 2011b). Hence, there is a need to evaluate species competition and turf quality dynamics on a mixed-stand where zoysiagrass and bermudagrass grow into each other.

In addition to AOPP herbicides, mesotrione, an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD), is reported to injury both common (Brosnan et al., 2011a) and hybrid bermudagrasses (Elmore et al., 2011). Unfortunately, it is unclear if the addition of mesotrione has a synergistic effect on AOPP herbicides. Additionally, there is a concern of the influence of traffic stress on bermudagrass control, as traffic is inconsistent across different parts of a golf course fairway. It is reported that zoysiagrass recovers slowly compared to bermudagrass under traffic stress (Trappe et al., 2011). Therefore, the objective of this research was to investigate the efficacy of the AOPP herbicides fenoxaprop and fluazifop in tank-mixes with triclopyr or mesotrione for control of common bermudagrass in a zoysiagrass fairway under low or high traffic locations.

Materials and Methods

Field experiments were conducted in 2010 and 2011 on the 9th fairway of The Falls Golf Club in O'Fallon, Missouri. This is a public course which averages 50,000 golf rounds each year. The fairway was established with 'Westwood' bermudagrass in 1994 and renovated to 'Meyer' zoysiagrass by sod in 2009, following three sequential applications of glyphosate applied four weeks apart. Shortly after sodding, bermudagrass was observed invading the fairway and becoming interwoven with zoysiagrass (Figure

3.1). The fairway soil was a Keswick silt loam with pH 6.7 and 4.5% organic matter. Field plots were established on two locations of the same fairway to represent high or low levels of traffic stress. A high traffic area (HTA) was located at the center of the fairway in front of the green, and a low traffic area (LTA) was situated on the left edge of the fairway adjacent to the rough. Both locations were in full sun and the topography was relatively flat. It was estimated that the LTA received only 20% of the traffic compared to the HTA. At both locations, plots 1.5 by 3 m were arranged in a randomized complete block design with four replications for the HTA, but only three replications for the LTA due to space limitation. The initial bermudagrass encroachments in plots at the two traffic areas varied from below 10% to above 40%. Plot areas were maintained by the golf course staff under typical fairway conditions excluding any herbicide applications.

Herbicide applications included fenoxaprop and fluazifop tank-mixed with either triclopyr or mesotrione (Table 3.1), and were applied to the same plots during the two-year study. Treatments with fenoxaprop also included a tank-mix with two rates of triclopyr at 0.23 and 0.85 kg ae ha⁻¹, with each designated as low and high rates, respectively. The rates and combinations were selected based upon previous greenhouse studies and the typical practices of local superintendents. In the first growing season, mesotrione tank-mixed with fenoxaprop or fluazifop resulted in $\geq 80\%$ or $\geq 60\%$ injury to zoysiagrass at 12 weeks after initial treatment (WAIT) for both traffic areas, respectively. Therefore, the two mesotrione-containing treatments were eliminated from the second year applications, and hence are excluded from discussion hereafter. Initial applications were applied on June 9, 2010 and June 8, 2011, when both bermudagrass and zoysiagrass were actively growing. The re-application intervals were not based on calendar intervals;

rather sequential applications were applied when approximately 30% of bermudagrass regrowth was observed in a majority of the treated plots. It is important that sequential applications are applied to green actively-growing tissue because the two AOPP herbicides are absorbed by leaves (Devine and Shimabukuro, 1994). Regrowth at 30% was chosen based upon the common standard that 80% or greater weed suppression is considered acceptable and 70% or less weed suppression is considered inadequate (Finn et al., 2008). Consequently, the re-application intervals were 3 weeks for the first three applications in 2010, and 2 weeks for the last two applications. In 2011, three applications were applied on 4 week intervals based upon bermudagrass regrowth (Table 3.1). All herbicide treatments were tank mixed with a non-ionic surfactant at 0.25% v/v, and applied with a CO₂-pressurized backpack sprayer calibrated to 206 L ha⁻¹ at a spray pressure of 276 kPa. Four TeeJet XR8002 flat fan nozzles (Spraying Systems Co., Wheaton, IL) at 38 cm spacing were used.

Visual assessment of percent bermudagrass coverage in each plot was rated regularly using a 0 to 100% scale, with 0% indicating no bermudagrass and 100% representing complete bermudagrass encroachment. For simplicity, only data collected at 0, 3, 8 and 12 WAIT are presented. To better represent season-long treatment efficacy on bermudagrass control, the area under percentage bermudagrass curves (AUPBC) was calculated. The AUPBC was calculated by the equation:

$$AUPBC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i) / 2] \times (t_{i+1} - t_i) \quad [1]$$

where X_i = percent bermudagrass coverage at the i^{th} observation, t_i = days after treatments at the i^{th} observation, and n = number of total observations (Campbell and Madden, 1990). The AUPBC value represents the cumulative effect throughout the entire

experiment, with a lower value indicating less bermudagrass encroachment, and hence more effective control.

Zoysiagrass injury, visible as chlorosis and stunted growth, was evaluated as a 0 to 100% scale, where 0% equals no visual injury symptoms and 100% indicates complete plant death. Normalized difference vegetation index (NDVI) was collected using the GreenSeeker[®] handheld sensor (NTech Industries, Ukiah, CA). The readings typically range from 0 to 1 on a green canopy, with increasing values corresponding to greater green canopy coverage and colors, and subsequently a higher turf quality (Bell et al., 2002; Trenholm et al., 1999).

Data analyses were conducted by PROC MIX of SAS 9.2 with repeated measurements (Kuehl 2000). Mean separation was conducted based on Fisher's Protected LSD ($P = 0.05$). ANOVA found that traffic was a significant factor interacting with other factors for all parameters. Therefore, data were presented separately for the HTA and LTA.

Results and Discussions

Effect of Traffic and AOPP Herbicide on Bermudagrass Control

Bermudagrass percent coverage at 0, 3, 8, and 12 WAIT were presented separately in both years after the initial applications (Table 3.2). During the two growing seasons, bermudagrass coverage in untreated plots increased 5- or 2-fold compared to the initial coverage in HTA and LTA, respectively (Table 3.2). AOPP herbicides tank-mixed with triclopyr significantly suppressed bermudagrass compared to untreated controls, and resulted in 81 to 100% bermudagrass reduction after the two-year program at both locations (Table 3.2). Although all treatments included in Table 3.2 showed a similar

efficacy at 12 WAIT, treatment with fluazifop tank-mixed with triclopyr resulted in complete bermudagrass removal after two-year applications at both locations. Increasing the rate of triclopyr appeared to improve the efficacy of fenoxaprop. In the first growing season, fenoxaprop tank-mixed with the high rate of triclopyr (0.85 kg ha^{-1}) significantly reduced bermudagrass coverage within 3 WAIT at both locations, which corresponds to one application. In comparison, fenoxaprop tank-mixed with the low rate of triclopyr (0.23 kg ha^{-1}) did not affect bermudagrass significantly until 8 WAIT, which corresponds to three sequential applications (Table 3.2). Lewis et al. (2010) reported that addition of triclopyr increased AOPP herbicide efficacy on bermudagrass control. Our results further indicate that increasing the triclopyr rate from 0.23 to 0.85 kg ha^{-1} is beneficial.

Another method to compare bermudagrass suppression among treatments is to examine the cumulative effect over the two growing seasons by calculating AUBPC. Our results showed that the most effective treatments were fenoxaprop tank-mixed with the high rate of triclopyr and fluazifop tank-mixed with triclopyr (Table 3.3). On the HTA, both of these treatments resulted in the lowest AUPBC, and were 12- or 11-fold lower than the untreated control, respectively. On the LTA, a similar trend was found, although the AUPBC values were generally smaller. Collectively, our data suggest that both fenoxaprop tank-mixed with the high rate of triclopyr or fluazifop tank-mixed with triclopyr were equivalent for control of bermudagrass. It is interesting to note that although the initial bermudagrass percent coverage in the untreated control plots were comparable at both locations (6.3% and 5.7% in HTA and LTA, respectively; Table 3.2), the AUPBC at the HTA is more than 2-fold greater than the AUPBC in the LTA (Table 3.3). This indicates that bermudagrass may be more difficult to control in high versus low

traffic environments on zoysiagrass fairways. Trappe et al. (2011) reported that bermudagrass exhibits better tolerance to traffic stress than zoysiagrass, supporting our result that traffic stress in HTA might provide a competitive advantage to bermudagrass over zoysiagrass.

Effect of Traffic and AOPP Herbicide on Zoysiagrass Injury and Overall Turf Quality

Despite the sequential applications, all treatments with AOPP herbicides tank-mixed with triclopyr caused < 5% zoysiagrass injury at 2 WAIT and no significant injury at 12 WAIT at both locations. These results agree with Lewis et al. (2010), who reported less than 7% visual injury to zoysiagrass following three sequential applications of fenoxaprop (0.14 kg ai ha⁻¹) tank-mixed with triclopyr (1.12 kg ae ha⁻¹) at monthly intervals. The same study also found minimal injury with a tank-mix of fluazifop (0.11 kg ai ha⁻¹) and triclopyr (1.12 kg ae ha⁻¹).

During this study, overall turf performance was evaluated by NDVI which provides an objective and quantitative evaluation of turf quality (Bell et al., 2002). A higher NDVI value indicates higher green vegetation coverage and darker green color, although it does not differentiate species in a mixed stand (Bell and Xiong, 2008). In this study, NDVI is a useful tool to detect potential chlorotic or thinning turf canopies despite the detected differences are due to herbicide injury to bermudagrass or phytotoxicity to zoysiagrass. Subsequently, NDVI measurements provide an overall assessment of the smoothness of transition from bermudagrass to zoysiagrass. In untreated plots, NDVI readings ranged between 0.7 and 0.9, except at 12 WAIT in the LTA (Figure 3.2). This result corresponds to an early report that a healthy, green turf canopy generates an NDVI

value around 0.8 or above (Xiong et al., 2007). The NDVI readings in treated plots overall followed the same trend of the NDVI collected from the untreated control plots. This indicates that the fluctuations in NDVI evaluation during the experiment period were mainly influenced by environmental or management factors, rather than treatment effects. Combined with visual assessment of zoysiagrass injury, NDVI values further demonstrate the minimal injury to zoysiagrass following herbicide applications. Among all treatments, fenoxaprop tank-mixed with triclopyr at the low rate (0.23 kg ha^{-1}) generated lower NDVI readings (Figure 3.2). The reduced NDVI values might reflect the herbicide injury on bermudagrass, as higher bermudagrass coverage were found in plots treated with fenoxaprop tank-mixing with triclopyr at the low rate (Table 3.2). However, compared to the untreated plots, NDVI readings from treated plots were either the same or less than 1 unit different in both locations. These results indicate that all treatment combinations maintained a smooth transition from bermudagrass to zoysiagrass during this experiment.

Conclusions

In summary, a two-year program with sequential applications of fenoxaprop or fluazifop tank-mixed with triclopyr can result in nearly 100% common bermudagrass control in a zoysiagrass fairway. Smooth and efficient transition can be achieved by applying fenoxaprop (0.12 kg ha^{-1}) in a tank-mix with triclopyr (0.85 kg ha^{-1}), or fluazifop (0.09 kg ha^{-1}) tank-mixed with triclopyr (1.0 kg ha^{-1}), with minimal injury to zoysiagrass. High traffic stress tended to favor bermudagrass growth over zoysiagrass and hence reduced treatment efficacy, but the reduction was minimal at the conclusion of the 2-year study. Tank-mixtures of AOPP herbicides with mesotrione caused

unacceptable injury to zoysiagrass and therefore should be avoided. Increasing the rate of triclopyr from 0.23 to 0.85 kg ha⁻¹ is beneficial, as it improves the efficacy and safety of fenoxaprop. In situations where bermudagrass and zoysiagrass are integrated, fenoxaprop and fluazifop were equally effective for bermudagrass control with adequate safety on zoysiagrass.

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Table 3.1. Treatment active ingredients, rates and application timings at various weeks after initial treatment (WAIT) applied to the two traffic areas on the 9th fairway of the Falls Golf Club in O’Fallon, MO in 2010 and 2011.

Trt	Herbicide active ingredient	Herbicide product	Active ingredient rate (kg ae or ai/ha) ^a	Product rate (Fl oz/acre)	Application timing (WAIT)	
					2010	2011
1	Fenoxaprop-p+ triclopyr (low)	Acclaim Extra + Turflon Ester	0.14 + 0.23	28.2 + 6.6	0, 3, 6, 8, 10	0, 4, 8
2	Fenoxaprop-p+ triclopyr (high)	Acclaim Extra + Turflon Ester	0.12 + 0.85	24 + 24	0, 3, 6, 8, 10	0, 4, 8
3	Fluazifop-p + triclopyr	Fusilade II + Turflon Ester	0.09 + 1.0	5.2 + 28.6	0, 3, 6, 8, 10	0, 4, 8
4	Mesotrione + fluazifop-p	Tenacity + Fusilade II	0.175+ 0.09	5 + 5.2	0, 3, 6, 8, 10	-- ^b
5	Mesotrione + fenoxaprop-p	Tenacity + Acclaim Extra	0.175+ 0.14	5 + 28.2	0, 3, 6, 8, 10	--
6	Untreated	--	--	--	--	--

^a Due to differences in herbicide chemistry, the triclopyr active ingredient rate is presented as acid equivalent per hectare, while other active ingredients are presented as active ingredient per hectare. Abbreviations: ae = acid equivalent; ai = active ingredient.

^b Mesotrione (treatments 4 and 5) were not applied in 2011 due to severe injury to zoysiagrass the previous year.

Table 3.2. Bermudagrass percentage coverage (%) at 0, 3, 8, and 12 weeks after initial treatment (WAIT) influenced by treatment at high traffic (HTA) or low traffic areas (LTA) in 2010 and 2011. There were significant interactions between location and evaluation dates; therefore, results were presented in different locations and evaluation times.

Trt	Herbicide	0 WAIT	3 WAIT	8 WAIT	12 WAIT
----- HTA in 2010 -----					
1	Fenoxaprop-p + triclopyr (low)	43.8 aA ^a	33.8 aA	13.7 aB	1.8 bC
2	Fenoxaprop-p + triclopyr (high)	13.8 bcA	2.5 bB	1.3 bB	0.5 bB
3	Fluazifop-p + triclopyr	18.8 bA	2.5 bB	1.0 bB	0.5 bB
6	Untreated	6.3 cA	13.7 bA	13.8 aA	15.0 aA
----- HTA in 2011 -----					
1	Fenoxaprop-p + triclopyr (low)	12.5 bA	4.3 bBC	6.3 bB	1.5 bC
2	Fenoxaprop-p + triclopyr (high)	3.3 cA	0.5 bA	0.5 cA	0.3 bA
3	Fluazifop-p + triclopyr	2.0 cA	0.8 bA	0.0 cA	0.0 bA
6	Untreated	21.3 aB	21.3 aB	25.0 aAB	27.5 aA
----- LTA in 2010 -----					
1	Fenoxaprop-p + triclopyr (low)	21.7 aA	13.7 aA	1.3 aB	1.6 aB
2	Fenoxaprop-p + triclopyr (high)	12.3 bA	2.7 bB	1.0 aB	0.6 aB
3	Fluazifop-p + triclopyr	3.7 bA	1.7 bA	1.7 aA	0.0 aA
6	Untreated	5.7 bA	6.7 abA	8.7 aA	8.3 aA
----- LTA in 2011 -----					
1	Fenoxaprop-p + triclopyr (low)	6.7 aA	3.7 abB	1.0 bC	0.0 bC
2	Fenoxaprop-p + triclopyr (high)	5.7 aA	2.0 bB	0.7 bB	0.0 bB
3	Fluazifop-p + triclopyr	2.0 bA	1.0 bA	1.0 bA	0.0 bA
6	Untreated	7.3 aA	6.0 aA	6.0 aA	10.0 aB

^a Means within each category in the same columns or rows labeled by the same lowercase or uppercase letters are not significantly different according to Fisher's Protected LSD ($P = 0.05$), respectively.

Table 3.3. Area under percent bermudagrass curve (AUPBC)^a at high traffic (HTA) or low traffic areas (LTA) in response to herbicide mixtures.

Trt	Herbicide	HTA	LTA
1	Fenoxaprop-p + triclopyr (low)	1998 b ^b	952 ab
2	Fenoxaprop-p + triclopyr (high)	296 c	315 b
3	Fluazifop-p + triclopyr	331 c	200 b
6	Untreated	3655 a	1562 a

^a Area under percent bermudagrass curve (AUPBC) was calculated by the equation [1] where X_i = percent bermudagrass coverage at the i^{th} observation, t_i = days after treatment at the i^{th} observation, and n = number of total observations. A lower AUPBC value means less bermudagrass encroachment and hence more effective control.

^b Means in the same column followed by the same letters are not significantly different according to Fisher's Protected LSD ($P = 0.05$).

Figure 3.1. Bermudagrass encroachment (darker areas) in zoysiagrass at the 9th fairway of the Falls Golf Club in O'Fallon, Missouri.



Figure 3.2. Normalized difference vegetation index (NDVI) readings collected at various weeks after the initial treatments (WAIT) affected by treatments at high traffic (HTA) or low traffic areas (LTA). A significant three way interaction of evaluation date by treatment by location was found and hence data were presented in individual figures with A: high traffic area in 2010; B: high traffic area in 2011; C: low traffic area in 2010; and D: low traffic area in 2011. Fisher's Protected LSD bars were presented as a: LSD for mean separation of different treatments; b: LSD for mean separation of different dates.

